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Iran J Parasitol

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Iranian Society of Parasitology
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Original Article

Detection and Molecular Characterization of Potentially Pathogenic Free-Living Amoebae from Recreational and Public Soils in Mazandaran, Northern Iran

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Received 23 Jul 2020
Accepted 19 Oct 2020

Keywords:
Free-living amoebae;
Soil;
Acanthamoeba spp.;
Naegleria;
Iran

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Abstract

Background: Free-living amoeba (FLA) belonging to *Acanthamoeba* spp., *Naegleria*, and *Balamuthia mandrillaris* are the soil-born protozoa. This study aimed to survey the occurrence of FLA, including *Acanthamoeba* spp., *B. mandrillaris*, *Vermamoeba* spp., and *Naegleria* spp., in soil samples collected from various districts of Mazandaran Province (Northern Iran) from July to December 2018.

Methods: Overall, 118 soil samples from the recreational and public places were surveyed for the existence of *Acanthamoeba* spp., *Vermamoeba*, *Naegleria*, and *B. mandrillaris* using both morphological key and molecular tools with genus-specific primers of JDP1, NA, ITS1, and Bal, respectively. To verify the taxonomic status of isolated amoeba, the phylogenetic tree was made based on sequences of 18S rRNA by MEGA (5.05) software with the maximum likelihood model.

Results: Overall, 61/118 samples (51.6%) were contaminated with FLA, and based on the sequencing data, 29 isolates were successfully sequenced. Among the samples, all isolated *Acanthamoeba* (52.4%) belonged to the T4 genotype with amplification of the DF3 region (18S rRNA gene). Internal transcribed spacer (ITS) sequencing revealed the presence of one strain of *Naegleria americana*. Twenty-eight *V. vermiformis* were also confirmed based on Nuclear SSU rDNA. Morphological survey and PCR assay did not show any positive samples for *B. mandrillaris*.

Conclusion: The present study indicates the occurrence of FLA in soil sources of the recreational and public places in Mazandaran province that it can be a severe risk to human health. Thus, more studies are expected to survey the infection source in patients with FLA-related diseases.